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PHYSIOLOGICAL AVAILABILITY OF THE VITAMINS: THIAMINE

By RUTH WOODS

Recent years have witnessed the perfection of new, improved methods for the detection and analysis of the vitamins in foods and biological materials. At the same time a greater insight into the biological action of the vitamins and the consequences of their deficiencies, individual or combined, has been achieved with respect to both animal and human organisms. Paralleling these advances in nutrition research, there has been an increasing awareness of the fact that the amount of a given vitamin present in the diet of the living organism as calculated by the food chemist may differ sharply from the amount which the organism actually utilizes for its respective biological needs. Consideration of this discrepancy between gross intake and net utilization of a nutrient is of primary importance in the accurate quantitative evaluation of a food or pharmaceutical preparation as a source of a given dietary essential. Based on this concept, a new field of nutrition investigation has grown up, largely during the past decade. This has been centered about methods for determining not only the vitamin content of diets *per se,* but the actual physiological availability of the vitamins in a given diet and the variations in vitamin availability under different conditions of diet and physiological status. Because of the almost infinite scope of this phase of nutrition research, the present Review will be limited to the discussion of investigations of the physiological availability of the vitamins to the human organism only. Studies with the water-soluble vitamin, thiamine, will be described in detail as an illustration of the findings which have been made or may be expected in this new field of research.

Vitamin Content vs. Availability:

There are many reasons why the calculated vitamin content of a given food or dietary supplement may not coincide with either the actual amount of the nutrient in the food, or the amount which is available for biological use by the body. These reasons may be catalogued, for the sake of convenience, into two major categories: (1) Those which reflect limitations inherent in the non-biological assay methods and (2) those which reflect interaction of dietary components or physiological or chemical processes with the ingested nutrient.

Limitations of Assay Procedures: Determination of the total vitamin content of a food can be made by physical, chemical or microbiological

methods. The choice of method depends on the nature and behavior of the test nutrient, as well as upon the medium in which the nutrient is carried. It is beyond the scope of this Review to describe the principles upon which these tests are based or to evaluate their individual advantages and disadvantages. Suffice it to say that any one of these methods, under certain conditions, may give rise to erroneous values or may be susceptible of misinterpretation even by the experienced analyst. The following examples, selected at random, will illustrate this point:

(Physical Methods): These methods depend largely on the measurement of the light absorbing or fluorescent properties of certain vitamins (e.g. carotene, vitamin A, riboflavin, pyridoxine). If a relatively pure substance is being analyzed, there is little chance for error. In natural foods, however, the possibility of irrelevant substances reacting like the test nutrient or interfering in its absorption curves must be considered. If corrections for these interfering factors are not made, values *either higher or lower* than the actual values may be obtained.

(Chemical Methods): Chemical methods may depend on color reactions or on titration procedures. Here again, irrelevant substances may erroneously accentuate the positive response; non-biological active forms of the vitamins may give a positive chemical reaction, while modified biologically active forms of the vitamin may fail to be detected. Here again are factors which are capable of contributing to overestimation or underestimation of the actual vitamin values.

(Microbiological Methods): Even the microbiological methods, while probably more specific than any of the physical or chemical assays, present possibilities for distortion of results. The microbiological test is based on the quantitative response of a living microorganism to the presence or absence of an essential nutrilite. Response is measured quantitatively in terms of lactic acid production (acidity measurements) or of growth (turbidity measurements). There are at least four possible ways in which erroneous values may be obtained: (1) Preliminary preparation of the test sample in order to obtain maximal yields often involves chemical procedures not always reproduced in the body (erroneously high values). (2) Other nutrients in the culture media may stimulate growth (fat, starch) or lactic acid production (glucose) irrespective of the test vitamin (erroneously high values). (3) Microorganisms may respond positively to compounds which are related to, or are precursors of, or are fragments of the vitamins. These compounds usually have no vitamin activity for the animal or human organism (erroneously high values). (4) Conversely, micro-

organisms may fail to measure vitamin derivatives which are active for animals and man (erroneously low values). With the exercise of proper precautions and the careful selection of test microorganisms, however, most of these sources of error can be eliminated. The question still remains (because of species differences): How closely do corrected microbiological values reflect the physiologically available vitamin content of foods?

Physiological Influences: The answer to the foregoing question must obviously come from studies which attempt to measure physiological availability in the animal organism itself.

(Biological Animal Assays): Animal assays are usually considered an accurate reflection of physiological availability—*in the species tested.*

However, as in the case of microbiological methods, the response may be determined by a combination of many factors. Results obtained with one species are not always applicable to other species and, in particular because of the artificiality of experimental conditions, may be even less applicable to human nutrition.

(Human Assays): Only when man is used as the test animal can a relatively accurate estimation of vitamin availability be expected. Studies involving man must take into account the innumerable physiological interactions which may alter the availability of a given quantity of a dietary vitamin. Such factors include variations in degree of vitamin absorption from the intestinal tract; release of "bound" vitamins from inactive protein complexes by digestive processes; alterations in vitamin requirement imposed by the presence or absence of other nutrients (other vitamins, amino acids, carbohydrates, etc.); alterations in requirement resulting from intestinal synthesis or destruction of the vitamins; the influence of anti-vitamins which may be co-existent in the diet.

Human Assay Methods:

During the past ten years numerous reports (1-13) have appeared concerning studies on the physiological availability of the vitamins in man. These have considered almost exclusively the water soluble vitamins, because the basis of the assay methods used is the measurement of the urinary excretion of these vitamins or of their derivatives. The property of the water solubility of these vitamins has a significant bearing on their net availability because they may be exposed, following ingestion, to a number of modifying influences such as enzymes, changes in pH, adsorbents, and others which operate most effectively in aqueous media. The water soluble

vitamins are excreted largely through the kidneys into the urine and, to a minor extent, through the sweat glands in perspiration. This fact serves as the basis for estimating physiological availability through measurements of the urinary excretions under varying conditions.

A Typical Urinary Excretion Method: In normal human subjects the urinary excretion of the water soluble vitamins has been found to be directly proportional to the amount consumed provided that, at the time of

Table 1. COMPOSITION OF BASAL DIET

Breakfast			
1 orange	150 g.	2 butter squares	14 g.
2 slices of toast (enriched)	50 g.	1 glass milk	230 g.
Dinner			
1 steak (lean)	150 g.	5 butter squares	35 g.
1 serving of fried potatoes	65 g.	1 glass of milk	230 g.
1 serving of carrots	60 g.	1 serving of apple pie	155 g.
1 serving of beets	70 g.	2 hard sugar candies	12 g.
2 slices of toast (enriched)	50 g.		
Supper			
2 fried eggs	90 g.	1 glass of milk	230 g.
1 lettuce serving	25 g.	1 apple	150 g.
1 tomato serving	70 g.	1 banana	150 g.
2 slices of bread (enriched)	66 g.	2 hard sugar candies	12 g.
4 butter squares	28 g.		

Analyses Conducted on Aliquots of the Composite Diet

Proximate analysis	Values found[1]	Vitamin content	Values found[1]
Total weight	2075 g.	Thiamine	1.34 mg.
Total solids	540 g.	Thiamine: Calorie ratio	0.5
Moisture	1535 g.	Thiamine: Non-fat calorie ratio	0.9
Protein	101 g.	Ascorbic acid	115 mg.
Fat (ether extract)	129 g.	Riboflavin	2.54 mg.
Ash	22 g.	Niacin[2]	22.0 mg.
Crude fiber	6 g.		
Carbohydrate (by difference)	282 g.		
Caloric value	2710 Cal.		
Non-fat calories	1550 Cal.		

[1] Expressed in terms of total food consumed in the 3 meals.

[2] Predominantly as the amide.

the tests, the subjects are subsisting on an adequate diet. There have been many modifications of the conditions under which collection and analysis of urinary samples have been made depending upon the individual investigators and their preferred methods. For purposes of simplicity and illustration this Review will describe in some detail a fairly typical human assay method recently developed by Melnick, Hochberg and Oser (9).

The general plan of this method is to compare the urinary excretion of a specific amount of the test vitamin when taken as part of the test dietary with the urinary excretion of the same quantity of vitamin when taken in pure aqueous solution—the form in which it is postulated to be most readily available for absorption. Extra quantities of the vitamin (or derivative) appear in the urine following dosage with the test material only after it has been extracted and absorbed from the gastrointestinal tract.* Using the basic concept that urinary vitamin excretion parallels the quantity consumed, the linear relationship of excretion to dosage is established for a group of experimental subjects by feeding graded doses of the vitamins in pure solution. These subjects must have first been shown to be in a state of good nutrition as evidenced by preliminary analyses of the urinary excretions. The test dose of the vitamin is taken immediately after the largest meal of the day. During this test period of two days, the subject eats a normal adequate basal diet of specific composition (Table 1).

The first 24-hour sample obtained *prior* to dosage is the reference or basal urine sample. The second 24-hour sample following the ingestion of the extra vitamin in pure solution is corrected for the basal excretion value of the previous day (Fig. 1, Page 6). To determine the physiologically available vitamin content of a test material, the experiment is repeated after an adjustment period of about two weeks. At that time the subject is given an amount of the test substance or food which has been calculated to furnish a critical dose of the vitamin involved. The ratio of the extra urinary excretion of the vitamin during this period to that following pure solution dosage with the same total quantity of the vitamin is the index of physiological availability. The precision and reliability of this method have been demonstrated by the authors (9, 14) and confirmed by other investigators as well (15, 16, 17).

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*Vitamins remaining in the gastrointestinal tract may be excreted in the feces. A major complicating factor in the appearance of vitamins in the feces has been found to be intestinal synthesis of these vitamins *(cf. Borden's Review of Nutrition Research,* May, 1946, "The Biosynthesis of Vitamins."). However, it has been demonstrated by several investigators that physiological availability studies may be conducted under controlled conditions so that any increase in vitamin content of the feces can be attributed to dietary vitamin unabsorbed from the gastrointestinal tract. When this is correlated with the failure of the vitamin to be excreted in the urine, the decreased availability of the nutrient can be definitely attributed to failure of absorption.

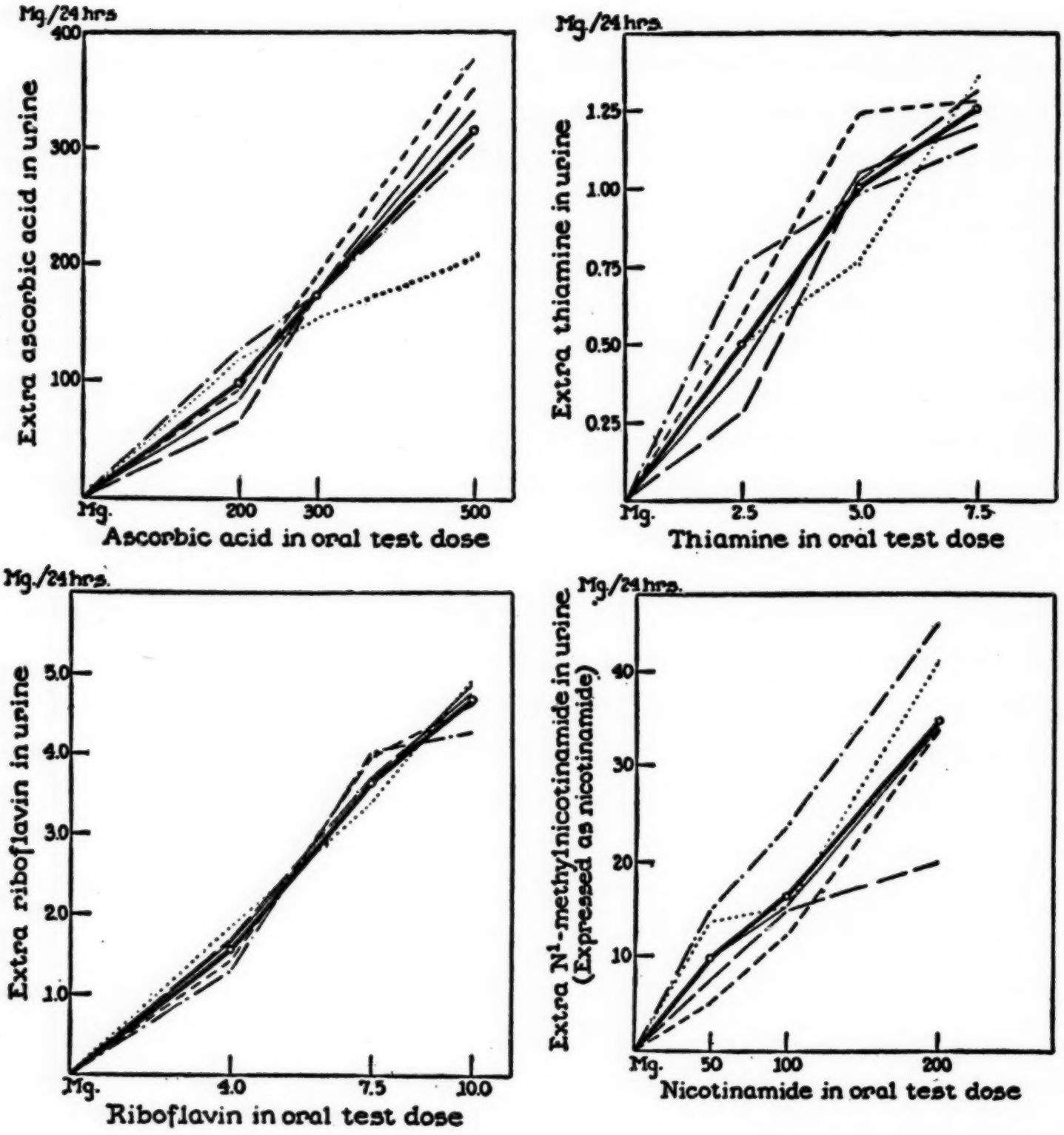

FIG. 1. The linear relationship between dosage with the water-soluble vitamins, ascorbic acid, thiamine, riboflavin and nicotinamide and the extra urinary excretion of the vitamins (or derivative). The test doses in aqueous solution (pH 3.0) were taken orally immediately after the mid-day meal. The fine lines represent the responses of the individual subjects whereas the heavy line indicates the average response.

Physiological Availability of Thiamine:

Using the human assay technic described, or similar procedures, it has been possible to obtain highly significant data on the physiological availability of the water-soluble vitamins as compared with the amounts of these vitamins actually provided in the diet. It has been possible to demonstrate not only availability under normal conditions, but also to estimate the effect of such factors as vitamin interrelationships, intestinal synthesis of vitamins, presence of anti-vitamins, and the like, in modifying the biological response to ingested vitamins.

The following description of some of the studies conducted with the vitamin, thiamine, best illustrates some of these findings.

Effect of Carbohydrate and Cellulose: The availability of dietary thiamine in man varies considerably, depending upon at least a half dozen or more factors. Thus, for example, increasing the amount of carbohydrate (8) in the diet has been shown to increase the requirement for thiamine, while an increase in the fat (4, 8) is apparently without effect.*

Increasing the intake of non-digestible cellulose fiber in the form of pineapple was found to have no effect on the urinary excretion of thiamine (18). The fecal excretion, however, was increased, suggesting that the fiber does not interfere with the absorption of dietary thiamine, but apparently actually stimulates intestinal synthesis of the vitamin. From the unchanged urinary excretions, however, it would appear that the intestinally synthesized vitamin is unabsorbed and, therefore, physiologically unavailable.

Effect of Adsorbents: The form in which thiamine is eaten appears to be another decisive factor in determining its availability in the human body. An interesting example of this is the early use of a fuller's earth adsorbate of a rice polish extract as the International Standard for vitamin B_1 (before crystalline thiamine had become available). Some years later it was shown by Keresztesy and Sampson (22) to be unsatisfactory as a reference standard because it really contained twice as much thiamine as was believed to be present; actually, the animal organism was able to extract only one-half of the vitamin held by the adsorbent.

Because adsorbing agents such as fuller's earth or kaolin are often taken as therapeutic agents in the treatment of various intestinal disorders, it is important to know what influence they may have on the availability of dietary nutrients. In an interesting study by Robinson *et al* (23) in 1940, it was noted that almost all patients with peptic ulcers receiving antacid medication and buffers of an adsorbent nature, such as magnesium trisilicate, excreted very small amounts of thiamine in the urine before and even after the taking of a test dose of thiamine. This led them to the subsequent demonstration that antacid medication and the presence of magnesium trisilicate were at fault, not the thiamine content of the diet (5). The creation of an alkaline reaction in the gastrointestinal tract favored greater destruction of thiamine, while the adsorbent (magnesium trisilicate) caused a loss of thiamine to the body by removing the vitamin from the digestive tract.

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*It is interesting to note that while studies on man indicate no effect of fat on thiamine requirement, animal experiments have demonstrated a marked reduction in the need for thiamine on a high fat diet (19, 20, 21).

In subsequent studies, Melnick *et al* (24) found that the biological effect of various adsorbents cannot always be predicted from the test-tube behavior of these materials. Thus, in an *in vitro* study comparing the behavior of fuller's earth and kaolin, both materials readily absorbed thiamine from aqueous solutions, the fuller's earth, however, showing a more marked affinity for the vitamin. Comparison of the effect of the two materials on thiamine by means of the human assay method, however, revealed a surprising difference. In the human assay study, the effect of fuller's earth was studied by administering a commercially available B-complex gelatin capsule containing fuller's earth.* A marked decrease in urinary excretion of thiamine was observed following the ingestion of these capsules as compared with an equivalent amount of thiamine in pure solution. *The availability of the thiamine in the commercial capsule was calculated to be only 27 per cent of the total amount of thiamine present in the capsule!*

Similar tests were made with a commercial preparation of kaolin. Kaolin is often used in the control of diarrhea because it regulates bowel action and adsorbs infectious gastrointestinal toxins which may be the cause of the diarrhea. Further, since prolonged diarrhea can result in an appreciable loss of nutrients, many kaolin preparations also contain B vitamins to replenish this loss. Unlike fuller's earth, kaolin was found not to diminish the availability of thiamine.

Effect of Live Yeast Cells: Although live compressed yeast has been widely acclaimed as a good source of B complex vitamins to such an extent that it became a dietary fad about 15 years ago, animal studies have indicated that the B vitamins are not readily available for absorption (25, 26). This is presumably due to the resistance of the live yeast cell to digestion, so that the vitamins are kept imprisoned within the cell and cannot be extracted for use by the body (27).

Investigation into the availability of thiamine and other B vitamins (chiefly riboflavin) from yeast for man were reported in 1940 (28) and 1942 (6, 29) from Parson's laboratory at the University of Wisconsin. It was found that the urinary excretion of thiamine by human subjects on a uniform diet to which four yeast cakes were added was more than twice as great when the yeast was boiled than when the yeast was eaten fresh. Parsons *et al* interpreted this to mean that thiamine is more completely assimilated from boiled yeast. Confirmation of this assumption was afforded by the fact that there was a much greater elimination of thiamine in the feces of the subjects fed live yeast than when they were fed an equal amount of fresh yeast which had first been boiled. This indicated that the

*As a riboflavin adsorbate

live yeast was unabsorbed from the digestive tract and eventually became lost through fecal excretion.

These studies were extended and confirmed several years later (11, 30) using several different strains of yeast. Not only were the live yeasts found to provide poorly utilizable thiamine, but the ingestion of these yeasts appeared to have even decreased the amount of available thiamine supplied by the basal diets by removing thiamine from the intestinal tract for use by the living yeast cells. This was deduced from the fact that the urinary thiamine was reduced below that of the preceding basal period and fecal thiamine was much increased. Furthermore, intact living yeast cells were recovered from the feces after fresh yeast feeding. Parson's studies were further confirmed by Hochberg *et al* (10) using the human assay technique.

Effect of Enzymes: About six years ago, Green *et al* (31) described an acute dietary deficiency disease of foxes (Chastek paralysis) caused by the feeding of certain types of raw fish. The disease, soon identified as a manifestation of thiamine deficiency, was found to be caused by the presence of a thiamine-destroying enzyme in the fresh fish (32, 33, 34). Although raw fish is not commonly employed in human dietaries, there are a number of smoked fish products intended for human consumption which do not undergo excessive heat processing, and there are some related foods (clams, oysters, salted herring and caviar) which may be eaten raw. The presence of the thiamine destroying enzyme, *thiaminase,* in clams was reported by Woolley (35). Subsequently studies were conducted by Melnick *et al* (36) to determine the presence of thiaminase in some of the products listed above and to ascertain the influence of this factor on the physiological availability of dietary and supplementary thiamine. From their *in vitro* studies they found that raw clams (chowder, steamer and cherrystone) destroy almost all of the thiamine present in a suspension of clams and thiamine; salted herring destroyed about 60 per cent of the thiamine present; marinated herring destroyed roughly about 50 per cent in some cases and only about 15 per cent in others; oysters, salmon caviar, smoked carp and smoked salmon exerted no thiaminase activity. Although raw carp is a good source of the thiamine destroying enzyme, the smoke-processing is apparently sufficient to destroy the anti-thiamine activity of the enzyme.

These experiments were followed by another series in which the effect of a product (raw clams) containing thiaminase was determined when fed to human subjects. The results, summarized in Table 2 on the next page indicate that about 50 per cent of the dietary thiamine was destroyed and that nearly half of the supplementary thiamine was destroyed as well.

Table 2. DESTRUCTION OF DIETARY AND EXTRA THIAMINE BY THIAMINASE IN CLAMS

Subject	Diet containing heated clams			Diet containing raw clams				
	Average basal excretion, mg./24 hrs.	Fraction of 7.5 mg. test dose excreted		Change in basal excretion[1]			Fraction of 7.5 mg. test dose excreted	
		1st 24 hrs., %	2nd 24 hrs., %	1st 24 hrs., %	2nd 24 hrs., %	3rd 24 hrs., %	1st 24 hrs., %	2nd 24 hrs., %
J. C.....	0.23	18.1	4.1	−46	−50	−56	10.3	0.8
E. M.....	0.21	14.8	1.5	−44	−56	−66	6.9	2.3
D. M.....	0.27	20.4	2.6	−36	−58	−60	8.4	1.9
M. H.....	0.22	10.8	2.2	−30	−37	−48	9.8	1.8
H. H.....	0.29	20.0	0.9	−53	−68	−68	11.4	1.2
Average....	0.24	16.8	2.3	−42	−55	−60	9.4	1.6
Average total excretion of extra thiamine		19.1%					11.0%	
Destruction of thiamine taken as test dose		$\frac{19.1 - 11.0}{19.1} \times 100 = 42\%$						

[1] Based on the average excretions of the corresponding subjects receiving the diet containing the heated clams.

Other Influences: Through the use of the human assay method and similar related techniques it has been possible to determine the influence of a wide variety of additional factors on the availability of thiamine. The method has been used, for example, to test the availability of vitamins in pharmaceutical tablets and capsules (14, 16, 37) particularly as it may be influenced by protective coatings, binders or similar constituents. It has also helped to point out that the intestinal synthesis of thiamine by man, while significant, is probably not a major factor in the thiamine economy of the human as it is in animals. Thiamine interrelationships with riboflavin have been observed with experimental animals in cases of severe deficiency of either of the two vitamins, but not under conditions of moderate dosage (38). This is an important consideration in evaluating the reliability of the technic used in the human assay method for determining the physiological availability of vitamins. In this method moderate doses of the test vitamins are administered and should, therefore, result in no interaction which would confuse the findings of urinary excretion analyses.

Conclusion:

Studies of the physiological availability of the vitamins to man have revealed that, in many cases, a significant difference may exist between the vitamin content of a food as estimated by the food chemist and the amount which is actually available for use by the human body. Numerous influences may be involved as factors in bringing about this discrepancy—the balance of other constituents of the diet, the form in which the vitamin is taken, concomitant occurrences of anti-vitamins, enzyme inhibitors, adsorbents, excess alkalis, etc., in the diet, occurrence of the vitamin as an unavailable portion of a natural protein complex, and others. In the light of these findings it seems likely that present practices of estimating dietary adequacy from tables of food values alone may have to be greatly revised to include evaluation of the many factors altering physiological availability of the vitamins if a true picture of vitamin intake is to be achieved.

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